

Worm-riding clam: description of *Montacutona sigalionidcola* sp. nov. (Bivalvia: Heterodonta: Galeommatidae) from Japan and its phylogenetic position

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Abstract

A new galeommatid bivalve, *Montacutona sigalionidcola* sp. nov., is described from an intertidal flat in the southern end of the Kii Peninsula, Honshu Island, Japan. Unlike other members of the genus, this species is a commensal with the burrowing scale worm *Pelogenia zeylanica* (Willey) (Annelida: Sigalionidae) that lives in fine sand sediments. Specimens were always found attached to the dorsal surface of the anterior end of the host body. This species has a ligament lithodesma between diverging hinge teeth, which is characteristic of *Montacutona* Yamamoto & Habe. However, it is morphologically distinguished from the other members of this genus in having elongate-oval shells with small gape at the posteroventral margin and lacking an outer demibranch. Molecular phylogenetic analysis based on the four-gene combined dataset (18S + 28S + H3 + COI) indicated that this species is monophyletic with *Montacutona*, *Nipponomontacuta* Yamamoto & Habe and *Koreomya* Lützen, Hong & Yamashita, which are commensals with sea anemones or *Lingula* brachiopods. This result suggests that host shifting across different phyla occurred at least twice in this clade.

Key words: ectocommensal, Galeommatidae, host shift, polychaete, scale worm, Sigalionidae, symbiosis

Introduction

The Galeommatidae *sensu* Ponder, 1998 is the most species-rich bivalve family, comprising about 620 described species and numerous undescribed species (Huber 2015). This family contains small to minute bivalves and exhibits high species diversity especially in tropical warm shallow waters (Bouchet *et al.* 2002; Paulay 2003; Lützen & Nielsen 2005). Many of them are commensals that live attached to host body surface or host burrow wall (Boss 1965; Morton & Scott 1989). The host taxa of the galeommatids are highly diverse as a whole, including crustaceans, annelids, echinoderms, cnidarians and brachiopods, although each galeommatid generally has high host specificity to particular host taxa (Boss 1965; Morton 1988; Morton & Scott 1989; Goto *et al.* 2012, 2014, 2018; Li *et al.* 2012).

Polychaetes are ones of the major host groups for galeommatids. *Neaeromya rugifera* (Carpenter, 1864) occurs in the respiratory cavity of the scale worm *Aphrodisia* spp., although it also occurs on the ventral surface of the mud shrimp *Upogebia pugettensis* (Dana, 1852) (Narchi 1969; Ó Foighil 1985; Li & Ó Foighil 2012). *Aligena elevata* (Stimpson, 1851) lives attached to the lower end of the tube of the bamboo worm *Clymenella torquata* (Leidy, 1855) (Gage 1968). *Kurtiella bidentata* (Montagu, 1803) has been collected from the burrows of the polychaetes, *Perinereis cultrifera* (Grube, 1840) and *Nereis irrorata* (Malmgren, 1867), although this species is also collected from burrows of various invertebrates (see the review of Ockelmann & Muus 1978). *Montacuta* sp. lives attached to the tube of the polychaete *Eunereis longissima* (Johnston, 1840) (Anthony 1916). *Kurtiella tumida* (Carpenter, 1864) lives within the exhalant halo of the host *Mesochaetopterus taylori* Potts, 1914 (Sendall *et al.* 1995). *Orobitella floridana* (Dall, 1899) and *Aligena* sp. are associated with the polychaete *Americanuphis magna* (Andrews, 1891) (Fox 1979). Two undescribed species were recorded as commensals with the polychaetes, *Notomastus lobatus* Hartman, 1947 and *Lepidasthenia varia* Treadwell, 1917, respectively, in the southeastern coast of the United States (Fox 1979; Ruppert & Fox 1988).

An undescribed galeommatid species is known to live attached to the body surface of the scale worm *Pelogenia zeylanica* (Willey, 1905) (Annelida: Sigalionidae), which burrows in fine sand sediments, in Kii and Boso Peninsulas, Honshu Island, Japan (Otani *et al.* 2005; Yanagi 2018). This species was tentatively assigned to the genus *Nipponomyssella* Yamamoto & Habe, 1959 (Otani *et al.* 2005), although it has not been described yet.

In this study, we described this species as *Montacutona sigalionidcola* sp. nov. based on the four specimens newly collected in Kamiura Beach, Kushimoto, Kii Peninsula, Japan. In addition, we performed molecular phylogenetic analyses to understand how a symbiotic association with *Pelogenia* Schmarda, 1861 evolved in Galeommatidae.

Materials and methods

Sampling and study sites. Field observations and sampling of galeommatids ectocommensal with *Pelogenia zeylanica* were made in Kamiura Beach, Kushimoto, Kii Peninsula, Japan (Figs. 1, 2), between 2000 to 2017. One additional observation was made at Tsubaki, Shirahama, Kii Peninsula, Japan (Fig. 1). We dug the soft sand sediments in the tidepool to about 20–50 cm in depth by using scoop shovels in order to collect *P. zeylanica*. If *P. zeylanica* harbored ectocommensal galeommatids, we recorded the number of bivalves per host. We collected four bivalve specimens from two individuals of *P. zeylanica* in Kamiura Beach on 3 May 2011 and 28 May 2017 for species description. Three specimens collected from the same individual of *P. zeylanica* in 2011 were preserved in a dry state and designated as paratypes, whereas a single specimen collected from *P. zeylanica* in 2017 was preserved in 99.5 % ethanol and designated as holotype. We photographed the latter in a living state with and without the host before preservation and used the foot tissue for molecular analyses.

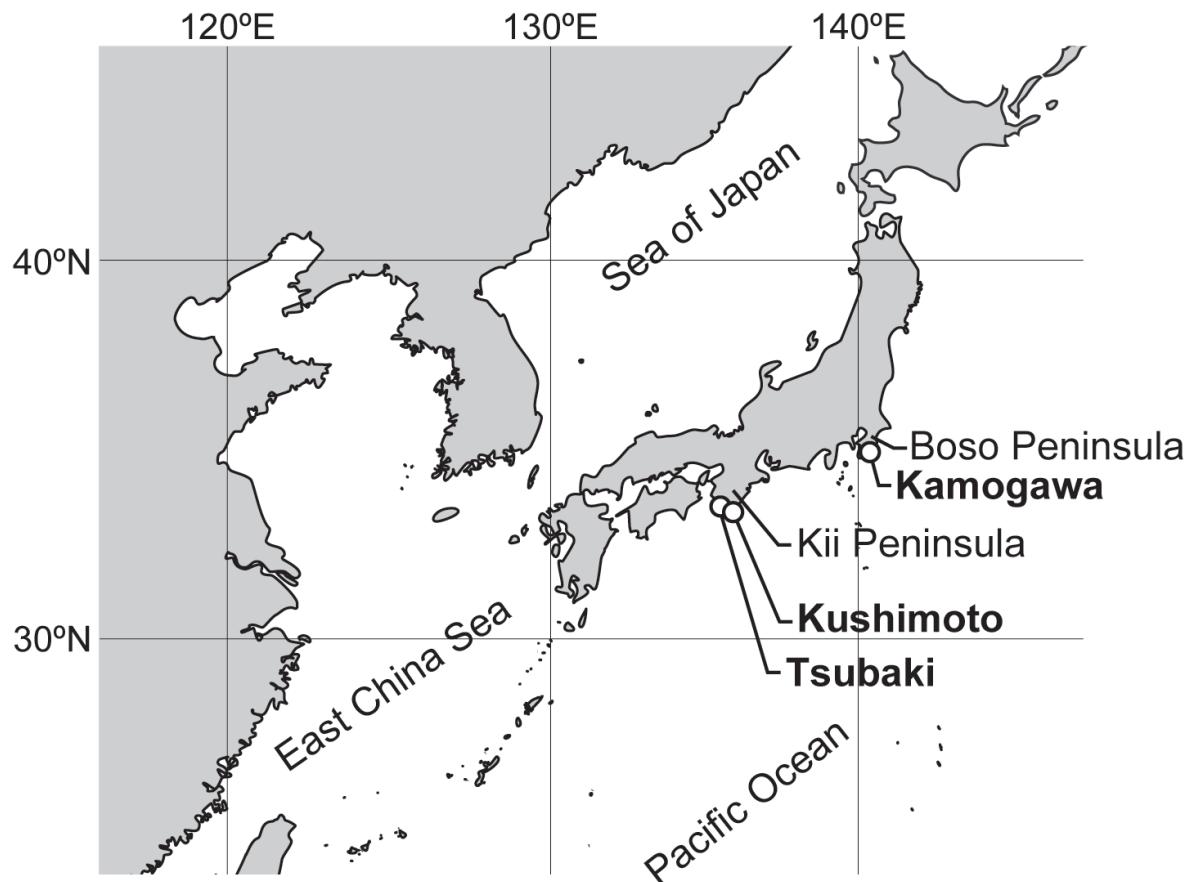


FIGURE 1. Sampling localities and previous records of *Montacutona sigalionidcola* sp. nov. Kushimoto (type locality), Wakayama prefecture, Japan (this study); Tsubaki, Shirahama, Wakayama Prefecture, Japan (this study; Otani *et al.* 2005); and Kamogawa, Chiba Prefecture, Japan (Yanagi *et al.* 2017).

Shell characteristics of these specimens and internal anatomy of the specimen collected in 2017 were observed under stereoscopic microscope. The hinge structure and nepioconch were taken by a scanning electron microscope (SEM, Real Surface View Microscope VE-9800, Keyence Corp.).

To investigate the phylogenetic position of this species within Galeommatidae, we performed molecular analyses based on the sequence data obtained in previous studies (mainly Goto *et al.* 2012, 2014) and those newly collected in this study (Table 1).

TABLE 1. List of species used for molecular phylogenetic analyses and their accession numbers. Accession numbers with an asterisk indicate the sequences obtained in this study.

| Family | Species | 18S rRNA | 28S rRNA | H3 | COI |
|---------------|--|-----------|-----------|-----------|-----------|
| Galeommatidae | <i>Anisodevonia ohshimai</i> | AB714754 | AB714797 | AB714838 | AB714878 |
| | <i>Arthritica japonica</i> | AB714755 | AB714798 | AB714839 | AB714879 |
| | <i>Basterotia carinata</i> | AB714780 | AB714823 | AB714861 | AB714902 |
| | <i>Basterotia gouldi</i> | AB714781 | AB714824 | AB714862 | AB714903 |
| | <i>Basterotia</i> sp. 1 | AB714782 | AB714825 | AB714863 | AB714904 |
| | <i>Borniopsis</i> aff. <i>ariake</i> | AB714776 | AB714819 | AB714858 | AB714899 |
| | <i>Borniopsis</i> aff. <i>nodosa</i> | AB714777 | AB714820 | AB714859 | AB714900 |
| | <i>Borniopsis macrophtalmensis</i> | AB714775 | AB714818 | AB714857 | AB714898 |
| | <i>Borniopsis ochetostomae</i> | AB714773 | AB714816 | AB714855 | — |
| | <i>Borniopsis subsinuata</i> | AB714774 | AB714817 | AB714856 | AB714897 |
| | <i>Borniopsis yamakawai</i> | AB714756 | AB714799 | AB714840 | AB714880 |
| | <i>Brachiomya stigmatica</i> | AB714753 | AB714796 | — | AB714877 |
| | <i>Curvemysella paula</i> | AB714757 | AB714800 | AB714841 | AB714881 |
| | <i>Devonia semperi</i> | AB714758 | AB714801 | AB714842 | AB714882 |
| | <i>Divariscintilla toyohiwakensis</i> | AB714745 | AB714788 | AB714831 | AB714869 |
| | <i>Entovalva lessonothuriae</i> | AB714759 | AB714802 | AB714843 | AB714883 |
| | <i>Ephippodonta gigas</i> | AB714746 | AB714789 | AB714832 | AB714870 |
| | <i>Galeomma</i> sp. 1 | AB714747 | AB714790 | AB714833 | AB714871 |
| | <i>Kellia porculus</i> | AB714760 | AB714803 | AB714844 | AB714884 |
| | <i>Koeanya setoensis</i> | AB907562 | AB907568 | AB907573 | AB907574 |
| | <i>Koreamya arcuata</i> | AB907557 | AB907563 | AB907569 | AB47955 |
| | <i>Kurtiella</i> aff. <i>bidentata</i> | AB714765 | AB714808 | — | AB714889 |
| | <i>Kurtiella bidentata</i> | KF741629 | KF741655 | — | — |
| | <i>Kurtiella pedroana</i> | — | KX376195 | KX375917 | — |
| | <i>Kurtiella tumida</i> | — | KX376206 | KX375841 | — |
| | <i>Lasaea undulata</i> | AB714761 | AB714804 | AB714845 | AB714885 |
| | <i>Litigiella pacifica</i> | AB714762 | AB714805 | AB714846 | AB714886 |
| | <i>Melliteryx puncticulata</i> | AB714763 | AB714806 | AB714847 | AB714887 |
| | <i>Montacutona sigalionidcola</i> | LC485247* | LC485248* | LC485249* | LC485250* |
| | <i>Montacutona</i> sp. 1 | AB714764 | AB714807 | AB714848 | AB714888 |
| | <i>Mysella charcoti</i> | KC429372 | KC429474 | KC429205 | — |
| | <i>Mysella</i> sp. 1 | — | KX376208 | KX375946 | — |
| | <i>Mysella vitrea</i> | AM774519 | AM779693 | KX375945 | — |
| | <i>Neaeromya rugifera</i> | AB714766 | AB714809 | AB714849 | AB714890 |
| | <i>Nipponomontacuta actinariophila</i> | AB714767 | AB714810 | AB714850 | AB714891 |
| | <i>Nipponomyssella oblongata</i> | AB714768 | AB714811 | AB714851 | AB714892 |
| | <i>Nipponomyssella subtruncata</i> | AB714769 | AB714812 | AB714852 | AB714893 |
| | <i>Paraborniola matsumotoi</i> | AB714770 | AB714813 | AB714853 | AB714894 |
| | <i>Peregrinamor gastrochaenans</i> | AB714771 | AB714814 | — | AB714895 |

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TABLE 1. (Continued)

| Family | Species | 18S rRNA | 28S rRNA | H3 | COI |
|-----------------|---------------------------------|----------|----------|----------|----------|
| | <i>Peregrinamor ohshimai</i> | AB714772 | AB714815 | AB714854 | AB714896 |
| | <i>Platomysia rugata</i> | LC126833 | LC126832 | — | — |
| | <i>Pseudogaleomma</i> sp. 1 | AB714748 | AB714791 | AB714834 | AB714872 |
| | <i>Pythina deshayesiana</i> | AB714778 | AB714821 | — | — |
| | <i>Salpocola philippinensis</i> | AB714779 | AB714822 | AB714860 | AB714901 |
| | <i>Scintilla aff. hydatina</i> | AB714750 | AB714793 | AB714835 | AB714874 |
| | <i>Scintilla rosea</i> | AB714749 | AB714792 | — | AB714873 |
| | <i>Scintilla</i> sp.1 | AB714751 | AB714794 | AB714836 | AB714875 |
| | <i>Scintilla</i> sp.2 | AB714752 | AB714795 | AB714837 | AB714876 |
| Outgroups | | | | | |
| Solecurtidae | <i>Azorinus minutus</i> | AB714783 | AB714826 | AB714864 | AB714905 |
| Gastrochaenidae | <i>Gastrochaena cuneiformis</i> | AB714784 | AB714827 | AB714865 | — |
| Veneridae | <i>Irus mitis</i> | AB714785 | AB714828 | AB714866 | AB714906 |
| Mactridae | <i>Meropesta nicobarica</i> | AB714786 | AB714829 | AB714867 | — |
| Trigoniidae | <i>Neotrigonia margaritacea</i> | AF411690 | DQ279963 | AY070155 | U56850 |
| Nuculanidae | <i>Nuculana pella</i> | AY070111 | AJ307553 | AY070148 | AY070138 |
| Solemyidae | <i>Solenmya velum</i> | AF120524 | AY145421 | AY070146 | U56852 |
| Solenidae | <i>Solen strictus</i> | AB714787 | AB714830 | AB714868 | AB714907 |

* Sequences obtained for this study are marked with an asterisk.



FIGURE 2. Kamiura Beach, Kushimoto, Wakayama Prefecture, Japan (type locality of *Montacutona sigalionidcola* sp. nov.)

Repository of type materials. The holotype and two paratype specimens are deposited in the National Museum of Nature and Science, Tokyo, Japan (NSMT; NSMT-Mo 79031–79033). One paratype is in the Smithsonian National Museum of Natural History, USA (USNM 1571983).

Molecular analysis. Total genomic DNA was isolated from the foot tissue of the bivalve specimen (NSMT-Mo 79031) using DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's protocol. Polymerase chain reactions (PCRs) were used to amplify 1384 bp of 18S, 1040 bp of 28S, 328 bp of H3 and 658 bp of COI. Amplification was performed in 25.23 µL mixtures consisting of 0.3 µL of forward and reverse primers (10 µM each; Table 2), 2.5 µL 10x ExTaq buffer, 2.0 µL dNTPs (2.5 µM each), 0.13 µL ExTaq polymerase (TaKaRa, Otsu, Japan), 17.5 µL of distilled water and 2.5 µL of genomic DNA. Thermal cycling was performed with an initial denaturation for 3 min at 94 °C, followed by 30 cycles of 30 s at 94 °C, 30 s at 49 °C (COI) or 55 °C (18S, 28S, and H3) and 2 min at 72 °C, with a final 3 min extension at 72 °C. PCR products were cleaned using ExoSAP-IT (Thermo Fisher Scientific, Tokyo, Japan). The sequencing reaction was performed in Eurofins Genomics (Tokyo, Japan) using PCR primers and internal primers (Table 2). The obtained sequences were deposited in the DDBJ/EMBL/GenBank databases with accession numbers LC485247–485250 (Table 1).

TABLE 2. Primers used for PCR amplification and sequencing in this study.

| Primer | Direction | Sequence 5'-3' | References |
|----------------------------------|-----------|------------------------------|--------------------------------|
| 18S rRNA | | | |
| PCR amplification and sequencing | | | |
| 1F | Forward | TACCTGGTTGATCCTGCCAGTAG | Giribet <i>et al.</i> (1996) |
| 3F | Forward | GTTCGATTCCGGAGAGGGA | Giribet <i>et al.</i> (1996) |
| 5R | Reverse | CTTGGCAAATGCTTCGC | Giribet <i>et al.</i> (1996) |
| 9R | Reverse | GATCCTTCCGCAGGTTCACCTAC | Giribet <i>et al.</i> (1996) |
| 18Sa2.0 | Forward | ATGGTTGCAAAGCTGAAAC | Giribet <i>et al.</i> (1996) |
| 18Sbi | Reverse | GAGTCTCGTTATCGGA | Giribet <i>et al.</i> (1996) |
| 28S rRNA | | | |
| PCR amplification and sequencing | | | |
| D1 | Forward | ACCCSCTGAA YT TAAGCAT | Colgan <i>et al.</i> (2003) |
| D3 | Reverse | GACGATCGATTGCACGTCA | Vonnemann <i>et al.</i> (2005) |
| Sequencing | | | |
| D2F | Forward | CCCGTCTTGAA AACAC CGGACCAAGG | Vonnemann <i>et al.</i> (2005) |
| C2R | Reverse | ACTCTCTCTCAAAGTTCTTTTC | Dayrat <i>et al.</i> (2001) |
| H3 | | | |
| PCR amplification and sequencing | | | |
| H3F | Forward | ATGGCTCGTACCAAGCAGACVGC | Colgan <i>et al.</i> (1998) |
| H3R | Reverse | ATATCCTTRGGCATRATRG TGAC | Colgan <i>et al.</i> (1998) |
| COI | | | |
| PCR amplification and sequencing | | | |
| LCO1490 | Forward | GGTCAACAATCATAAAGATATTGG | Folmer <i>et al.</i> (1994) |
| HCO2198 | Reverse | TAAACTTCAGGGTACCAAAAAATC | Folmer <i>et al.</i> (1994) |

Sequences of the 18S, 28S, H3, and COI genes were aligned using the program Muscle (Edgar 2004) as implemented in the software Seaview (Galtier *et al.* 1996; Gouy *et al.* 2010) with default settings; poorly aligned regions were corrected by eye. The alignments of H3 and COI sequences (328 and 658 bp, respectively) had no indels and were therefore unambiguous. We employed Gblocks v0.91b (Castresana 2000; Talavera & Castresana 2007) to eliminate ambiguously aligned regions for 18S and 28S datasets. Lengths of sequences prior to and following the Gblocks treatment were 2029 and 1724 (18S) and 1352 and 920 (28S), respectively.

Phylogenetic trees were constructed based on the combined dataset (18S + 28S + H3 + COI) using Bayesian inference and maximum likelihood (ML) methods. The Bayesian analysis was performed using MrBayes 3.2.5 (Ronquist & Huelsenbeck 2003) with substitution models chosen by Kakusan 4 (Tanabe 2011) for each gene and codon position [18S: K80_GAMMA, 28S: GTR_GAMMA, H3: GTR_GAMMA, GTR_GAMMA, and JC69_Homogeneous (for each codon), COI: HKY85_GAMMA, GTR_GAMMA, and GTR_GAMMA (for each codon)]. Two independent runs of Metropolis-coupled Markov chain Monte Carlo were carried out simultaneously for 4,000,000 generations, sampling trees every 100 generations. The initial 25% of the sampled trees were discarded as burn-in. We confirmed that analyses reached stationarity well before the burn-in period both by checking the ASDSFs and plotting the ln-likelihood of the sampled trees against generation time.

Maximum likelihood (ML) analysis was performed using RAxML (Stamatakis 2014) as implemented in raxml-GUI 1.5 (Silvestro & Michalak 2012). Datasets were partitioned by gene and codon position and GTR + GAMMA model was implemented for all partitions. Robustness of tree topology was evaluated by bootstrap support values (BS) from 1,000 replications. We mapped the host taxa and host use pattern on a part of tree to show how *M. sigalionidcola* evolutionarily achieved the association with *Pelogenia*. The information of the host taxa and host use pattern is based on Goto *et al.* (2012), Goto *et al.* (2014) and Li *et al.* (2016).

Systematics

Superfamily Galeommatoidea J.E. Gray, 1840

Family Galeommatidae *sensu* Ponder, 1998

Genus *Montacutona* Yamamoto & Habe, 1959

Diagnosis. Shell typically suborbicular in shape with submedian umbones. Two pronounced and spread laterals in left and right valves. A calcified, white portion (*i.e.*, lithodesma) situated in-between the spread laterals. Outer demibranch significantly reduced or absent.

Montacutona sigalionidcola sp. nov.

[Japanese name: Sunaurokomushi-yadori-gai]

Figs. 3–5

Nipponomyssella sp. Otani *et al.* 2005: 73, pl. 5, fig. 5
Sunaurokomushi-yadori-gai Yanagi 2017: 10

Material examined. Holotype (Figs. 3, 4): NSMT-Mo 79031 (SL 3.9 mm, SH 2.6 mm, Kamiura Beach, 28 May 2017). Paratype 1 (Fig. 5A): NSMT-Mo 79032 (SL 4.8 mm, SH 3.5 mm, Kamiura Beach, 3 May 2011), paratype 2 (Fig. 5B): USNM 1571983 (SL 4.9 mm, SH 3.1 mm, Kamiura Beach, 3 May 2011), paratype 3 (Fig. 5C): NSMT-Mo 79033 (SL 2.9 mm, SH 1.9 mm, Kamiura Beach, 3 May 2011).

Type locality. Kamiura Beach, Kushimoto, Wakayama, Kii Peninsula, Japan (33°27'N, 135°46'E; Figs. 1, 2).

Habitat. Intertidal sand (Fig. 2).

Diagnosis. Shell ovate-elongate in shape with a small gape at posteroventral margin. Only inner demibranch present.

Description. Adult shell (Figs. 3–5) small (up to 4.9 mm), thin, fragile, anteroposteriorly ovate-elongate, dorsally subtrigonal, equivalve, inequilateral. Shell inflated posteriorly. Umbo prominent; beak opisthogryrate, located slightly posterior to mid-length of shell. Shell color pale yellowish but slightly whitish and polished around the beak. Height/length about 0.73–0.63.

Anterior shell margin rounded; posterior margin roundly subtruncate; anterodorsal margin straight; posteroventral margin nearly straight but slightly incurved near umbo; posteroventral margin nearly straight. Shell margins meeting at the all points except for small gape along the posteroventral margin (Fig. 3C). Byssal threads extending from the base of the foot through the posteroventral gape of the shell to the outside. Shell sculpture consisting of many closely-set commarginal growth lines and evenly spaced fine radial ribs.

Hinge of left valve consisting of thickened marginal lateral teeth, diverging approximately 90° (Fig. 4E). Hinge of right valve consisting of distinct lamella-like teeth, diverging approximately 90° (Fig. 4F). Resilium (internal ligament) on left and right valves located immediately below umbo and between anterior and posterior tooth, with a rounded to trapezoid lithodesma anteriorly slightly curved (Fig. 4E, F). No external ligament.

Prodissococonch I and II indistinct. The nepioconch/adult shell boundary with SL = 1220 µm, SH = 612 µm in holotype (Fig. 4G, H)

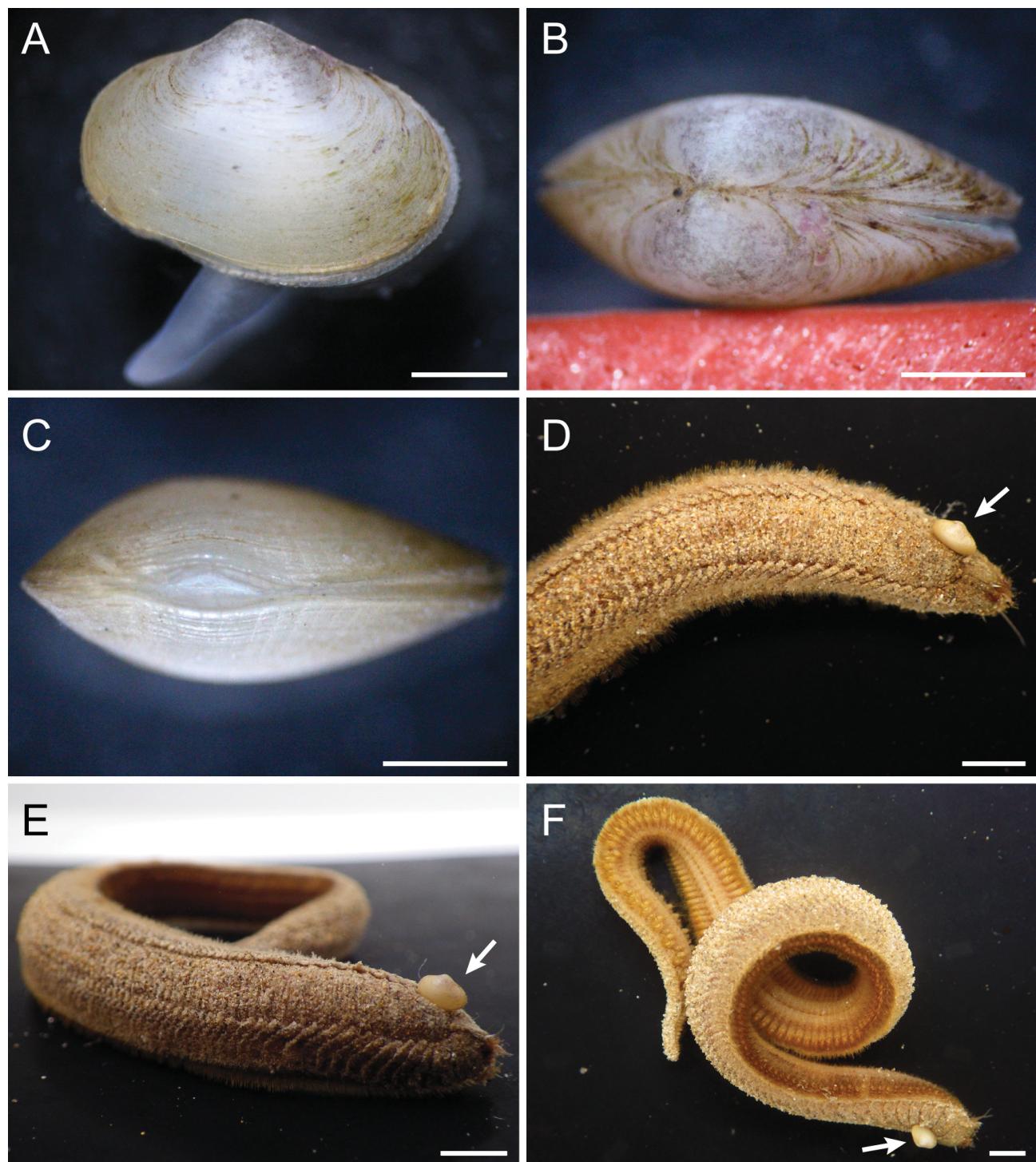


FIGURE 3. *Montacutona sigalionidcola* sp. nov. (holotype, NSMT-Mo 79031, SL 3.9 mm) and its host *Pelogenia zeylanica*. (A) A crawling individual of *M. sigalionidcola*. (B, C) Dorsal and ventral sides of *M. sigalionidcola*. (D–F) *Pelogenia zeylanica* with *M. sigalionidcola* attached (arrowed). Scale bar: 1 mm (A–C), 5 mm (D–F). Photo credits: R. Goto (A–F).

Soft parts: Mantle not reflected, without tentacles. Mantle edge narrowly extending beyond margin of shell and somewhat rugose (Fig. 3A). Both anterior and posterior adductor muscles elongate ovate and located in relatively dorsal position (Fig. 4C, D). Ctenidia consisting of inner demibranch with ascending and descending lamellae (Fig. 4C, D). Labial palps small (Fig. 4C, D). Foot slender, laterally compressed, rounded in front (Fig. 3A). Byssal gland located at the base of the foot.

Distribution. Kushimoto (this study) and Tsubaki (Otani *et al.* 2005; this study), Kii Peninsula, Wakayama Prefecture, and Uchiura, Kamogawa, Boso Peninsula, Chiba Prefecture, Japan (Yanagi 2017) (Fig. 1).

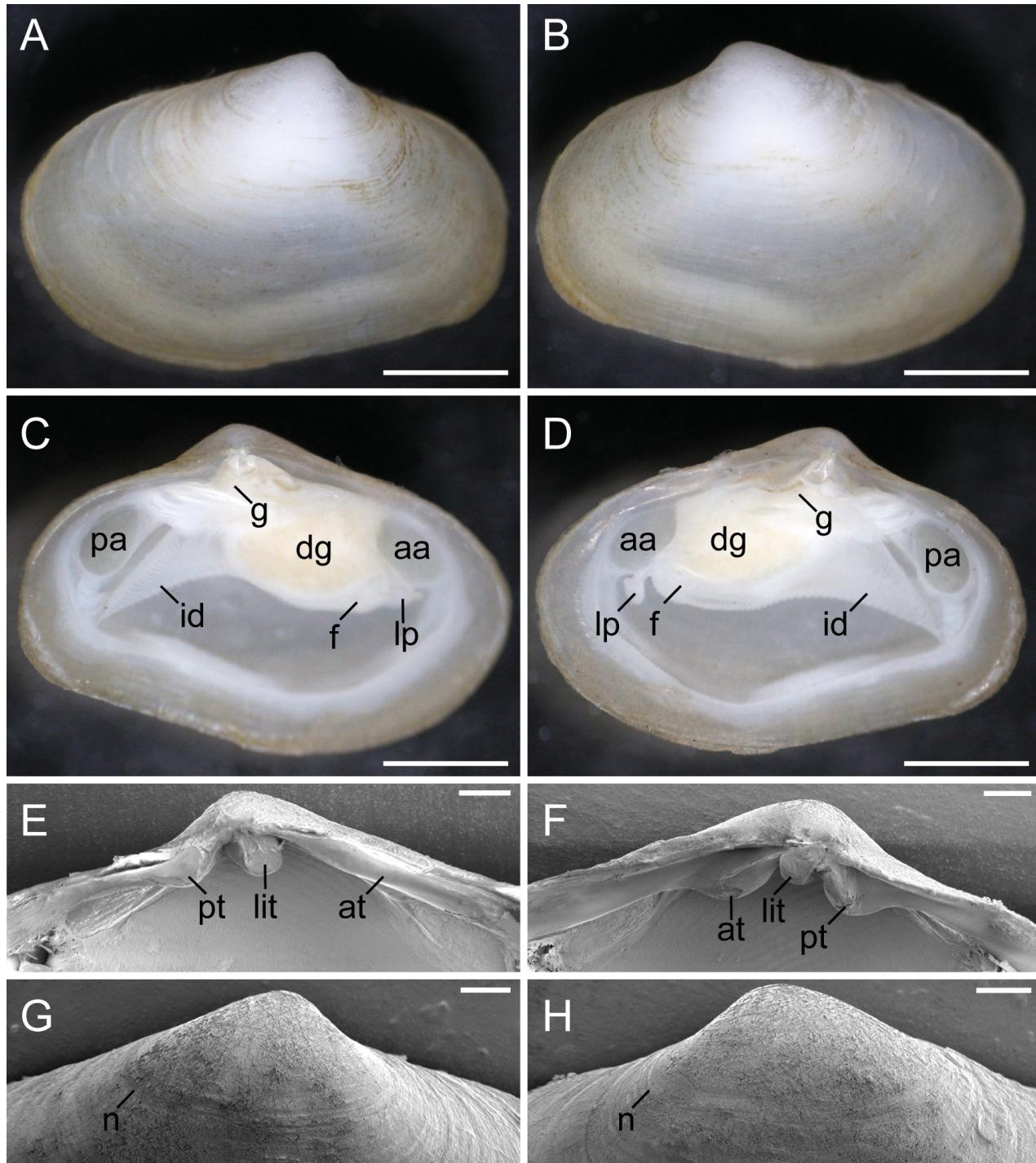


FIGURE 4. Holotype of *Montacutona sigalionidcola* sp. nov. (NSMT-Mo 79031, SL 3.9 mm). (A, C) Left valve. (B, D) Right valve. The foot was removed for DNA analysis. (E, F) Hinge structure of left and right valves. (G, H) Umbos of left and right valves. Abbreviations: aa, anterior adductor muscle; at, anterior lateral tooth; dg, digestive gland; f, foot; g, gonad; id, inner demibranch; lp, labial palp; lit, lithodesma; n, nepioconch-adult shell boundary; pa, posterior adductor muscle; pt, posterior lateral tooth. Scale bar: 1 mm (A–D), 200 µm (E–H). Photo credits: R. Goto (A–H).

Host. *Pelogenia zeylanica* (Annelida: Sigalionidae) (Fig. 3D–F).

Host association. This species is tightly attached to the dorsal surface of the anterior end of the host by byssal threads (Fig. 3). The anterior end of the holotype was directed towards the anterior end of the host. The number of *M. sigalionidcola* per host ranged from one to four (Table 3). The infestation rate by *M. sigalionidcola* is high: roughly 80% of *P. zeylanica* harbored commensal bivalves (Tanaka, personal observations).

Phylogenetic analysis. Figure 6 shows the phylogenetic position of *M. sigalionidcola* in Galeommatidae. This species forms a monophyletic group with *Montacutona*, *Nipponomontacuta*, and *Koreamya* [Bayesian posterior probabilities (PP) = 1.00, ML bootstrap rates (BS) = 99].

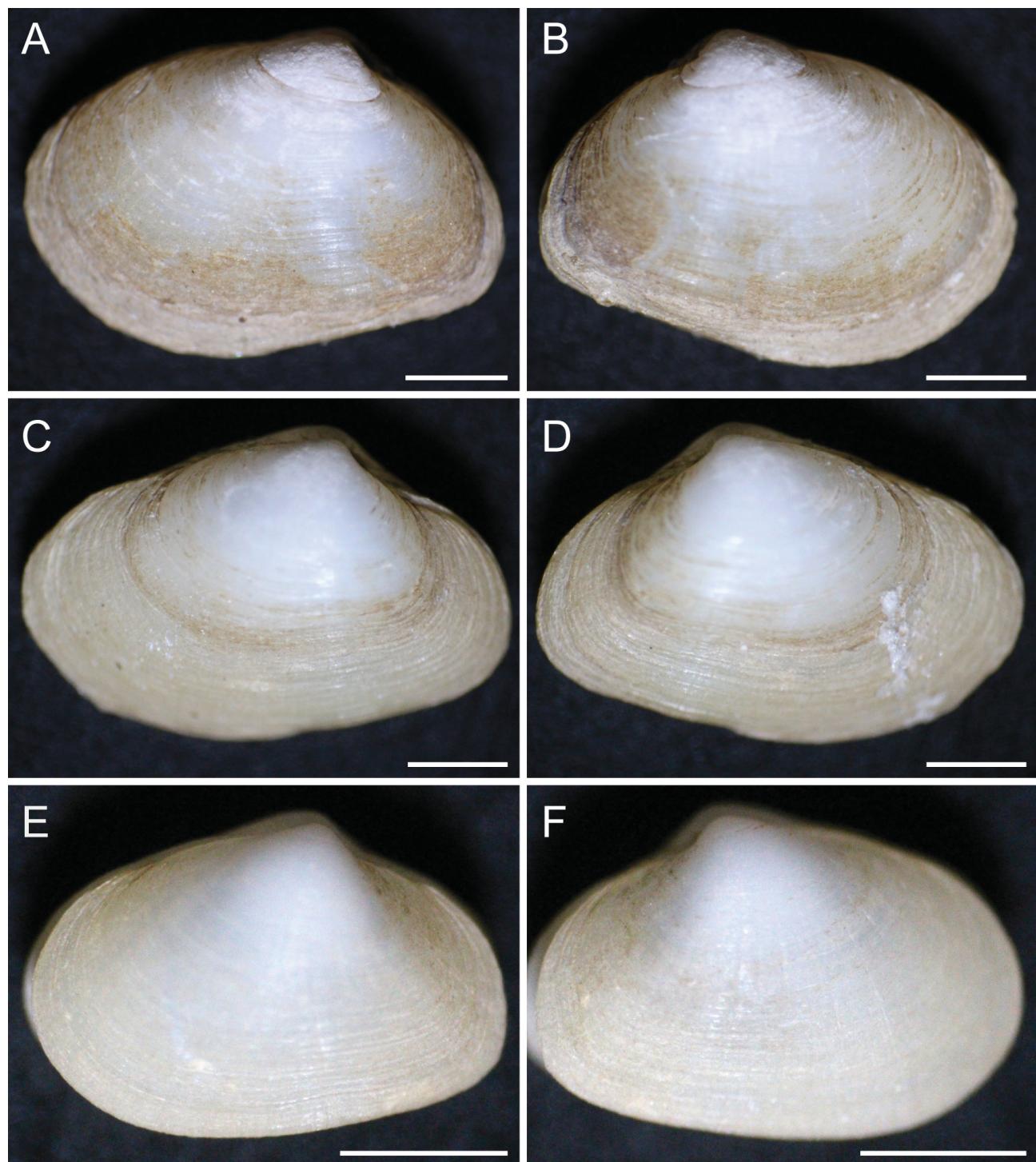


FIGURE 5. Paratypes of *Montacutona sigalionidcola* sp. nov. (A, B) Left and right valve of paratype 1 (NSMT-Mo 79032, SL 4.8 mm). (C, D) Left and right valve of paratype 2 (USNM 1571983, 4.9 mm). (E, F) Left and right valve of paratype 3 (NSMT-Mo 79033, 2.9 mm). Scale bar: 1 mm. Photo credits: R. Goto (A–F).

TABLE 3. Number of *Montacutona sigalionidcola* per host with its sampling localities and sampling dates.

| Date | Sampling locality | Number of individuals per host |
|--------------|---|--------------------------------|
| 15 May 2000 | Tsubaki, Shirahama, Wakayama, Japan | 2 |
| 20 July 2008 | Kamiura Beach, Kushimoto, Wakayama, Japan | 4 |
| 3 May 2011 | Kamiura Beach, Kushimoto, Wakayama, Japan | 3 (paratypes) |
| 4 June 2011 | Kamiura Beach, Kushimoto, Wakayama, Japan | 2 |
| 7 June 2015 | Kamiura Beach, Kushimoto, Wakayama, Japan | 4 |
| 10 June 2017 | Kamiura Beach, Kushimoto, Wakayama, Japan | 1 (holotype) |

Etymology: The species name is derived from the family name of the host and the suffix *cola* (Latin), meaning a dweller or inhabitant.

Discussion

The genus *Montacutona* Yamamoto & Habe, 1959 comprises about ten species, although the species border is often difficult to determine (Huber 2015). The biology of many members of this genus remains unknown, but several species (e.g., *M. mutsuwakensis* Yamamoto & Habe 1959 and *M. ceriantha* Ponder, 1971) are commensal with burrowing sea anemones of *Cerianthus* Delle Chiaje, 1841 (Ponder 1971; Morton 1988; Morton & Scott 1989), whereas *M. compacta* (A. A. Gould, 1861) is free-living or perhaps commensal with corals (Morton 1980). Our finding adds a new commensal species to this genus.

The members of the genus *Montacutona* have a calcified lithodesma between diverging lateral tooth (Morton 1980; Huber 2015). *Montacutona sigalionidcola* also has such a lithodesma. Most species of *Montacutona* have a reduced outer demibranch (Morton 1980; Huber 2015), whereas *M. sigalionidcola* lacks an outer demibranch. Shell shape of *Montacutona* is rounded to trigonal (Huber 2015), whereas that of *M. sigalionidcola* is ovate-elongate. In addition, *M. sigalionidcola* is distinguished from other members of this genus in having a small gape along the posteroventral margin. *Montacutona sigalionidcola* attaches strongly to the host body surface by byssal threads, which are extended through this small gape. Thus, the small gape of the shell is considered to be a morphological specialization to their ectocommensal lifestyle.

Montacutona sigalionidcola was previously treated as *Nipponomyssella* sp. (Otani *et al.* 2005) probably because this species has an elongated oval shell. However, molecular phylogenetic analyses in the present study suggest that *M. sigalionidcola* is not monophyletic with *N. oblongata* (Yokoyama, 1922), the type species of *Nipponomyssella* (Fig. 6), but is monophyletic with *Montacutona*, *Nipponomontacula* Yamamoto & Habe, 1959 and *Koreanya* Lützen, Hong & Yamashita, 2009 (Fig. 6). Considering that *Koreanya* and *Nipponomontacula* also have a lithodesma between diverging lateral tooth and a reduced outer demibranch (Goto *et al.* 2014), it may be reasonable that these genera are synonymized with *Montacutona*.

Montacutona sigalionidcola attaches only to the dorsal surface of the anterior end of the host scale worm. Many ectocommensal galeommatids are known to have a similar attachment-site specificity (Kato & Itani 1995; Goto *et al.* 2014, 2018). It has been suggested that the attachment site allows the commensal to effectively receive water currents created by the hosts and use them for filter feeding and respiration. This may also be applied for *M. sigalionidcola*. On the other hand, *Pelegenia zeylanica* autotomizes a body part when it is shocked (Goto, personal observations). By attaching to the anterior end of *P. zeylanica*, the bivalves may reduce the risk of being left behind during an autotomizing event.

Montacutona sigalionidcola has been recorded only from Kii and Boso Peninsulas, Honshu Island, Japan (Fig. 1). Considering that the host scale worm *P. zeylanica* has also been recorded from more southern localities (e.g., Iriomote Island and Izu Oshima Island, Japan, and Indonesia) (Imajima 2007), *M. sigalionidcola* also may occur at those localities.

Host shift is a common speciation process in symbiotic and parasitic organisms (Coyne & Orr 2004). Host shifts revealed by molecular phylogenetic analyses occurred mostly between closely related host taxa (e.g. Hafner & Nadler 1988; Shaw 1988; Faucci *et al.* 2006; Tsang *et al.* 2009). However, recent molecular phylogenetic analyses of Galeommatidae suggest that host shifts across distantly related host taxa (e.g. phyla) are common in some clades (Goto *et al.* 2012, 2014; Li *et al.* 2016). In the present study, *M. sigalionidcola*, a commensal with a sigalionid scale

worm, forms a monophyletic group with commensal species associated with sea anemones and species associated with *Lingula* brachiopods (Fig. 6). This suggests host shifts across phyla occurred at least twice in this clade. Sigalionid scale worms, burrowing sea anemones, and *Lingula* brachiopods are ecologically similar in inhabiting fine sand sediments. This suggests that the habitat preference for specific sediments type may more strongly constrain the diversification of this galeommatid clade than preference for specific host taxa. Our analysis included *Neaeromya rugifera*, which is also known as an ectocommensal of the scale worm, *Aphrodita* spp. (Li & Ó Foighil 2012). They were not monophyletic in the phylogenetic tree (Fig. 6), suggesting that ectocommensal associations with scale worms evolved at least twice in the Galeommatidae.

Sea anemones and *Lingula* brachiopods, the hosts of *Montacutona*, *Nippomonmontacuta*, and *Koreamya*, are nearly sessile animals. In contrast, sigalionid scale worms, the host of *M. sigalionidcola*, are much more mobile. Shells of *M. sigalionidcola* are dorsoventrally flatter than those of other *Montacutona*. Perhaps, it is an adaptation to reduce the friction against sediments around the hosts when it moves sediments.

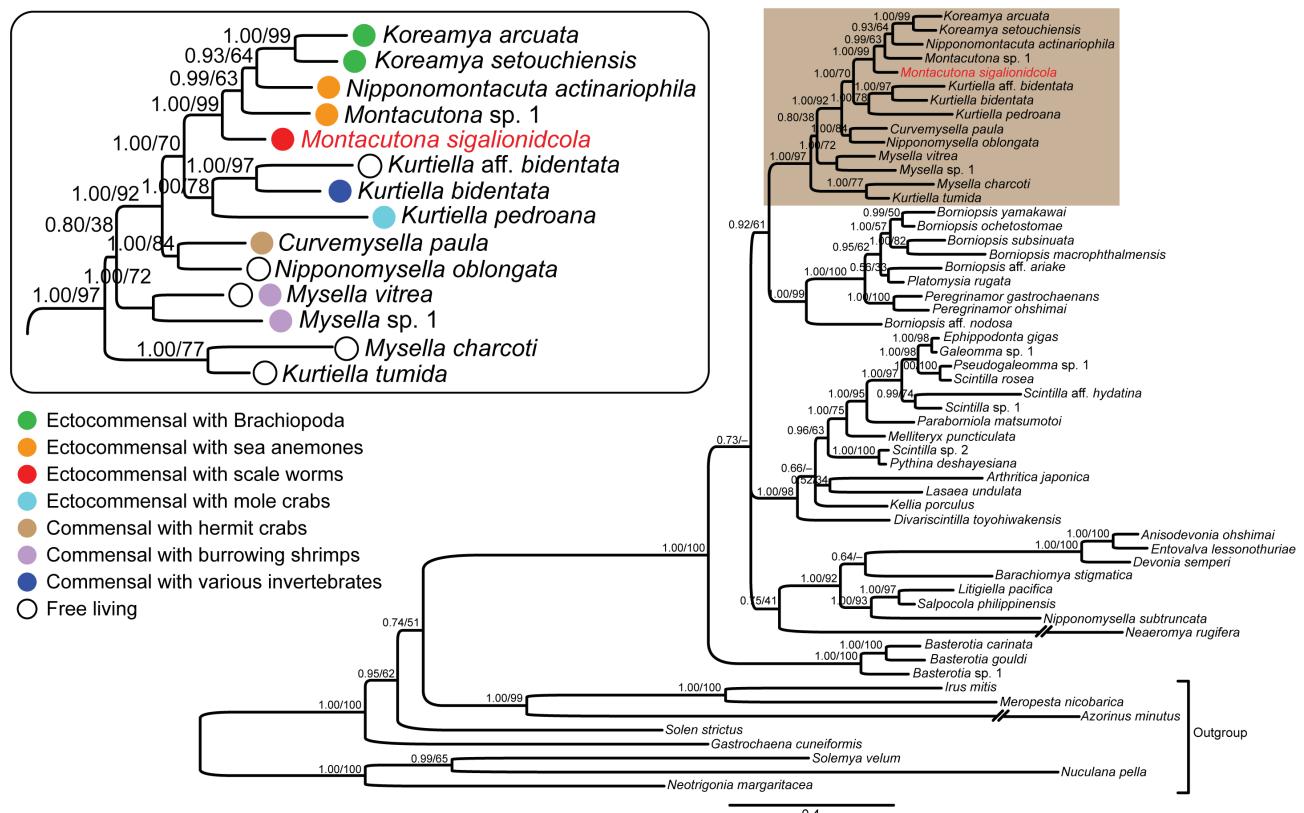


FIGURE 6. Bayesian phylogenetic tree of Galeommatidae, including *Montacutona sigalionidcola* sp. nov., based on the combined dataset of 18S, 28S, H3 and COI genes. Numbers above branches indicate Bayesian posterior probabilities followed by maximum likelihood bootstrap support values. A part of the galeommatid tree is closed up to show evolutionary pattern of host associations.

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